INVESTIGATION OF COMPLEMENT CONTROL MODULES BY NMR-SPECTROSCOPY

Ph.D. theses

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1. Introduction

The regulated activation of the complement system of an organism plays an important role in defense against pathogens, in preserving its homeostasis. The activation of the classical pathway, one of the three pathways of the system, is realized in the C1 complex via the autoproteolysis of the C1r serine protease. X-ray crystallographic studies provided valuable structural information on the components of the complex (C1r, C1s and C1q), nevertheless the interactions and especially their internal dynamics are not well known.

With the improvement of NMR-spectroscopy in the last decades, we may apply a unique tool to give dynamic pictures of protein solutions complementing the highly static view of crystallography. These studies are able to detect not only weak protein-protein interactions masked and therefore unobserved by crystallography but also the motions of bonds through the relaxation behavior of atoms providing valuable pictures of proteins. On the basis of the wealth of dynamic information available, the motions on characteristic time scales of protein regions may have highly important functional roles. Detection of such regions may promote to the understanding of protein-protein interactions significantly.

The crystal structures (PDB: 1gpz and 2qy0) of the catalytic fragment (CCP1-CCP2-SP) are hardly give explanations on the activation mechanism of C1r zymogen, which is even more difficult in the case of the inactive dimer (1gpz). The activation should accompanied by a large conformational rearrangement disrupting the well-defined $CCP1_A$:SP_B interaction. The molecule encompasses two CCP modules providing further catalytic curiosity. The SP covalently linked to CCP2 has one magnitude increase in cleavage of its C1s substrate, the phenomenon is decreased by the further presence of covalently linked CCP1.

The structure-dynamics studies of CCP modules are not without precedent, thus these data may help in characterization of C1r CCP behaviors. Understanding of the dynamics and interactions of C1r CCP modules is therefore may assist considerably to connect structure and function of C1r, or even of C1 complex, to the deeper insight into classical pathway.

2. Objectives of research

The aims were to characterize the solution structure of the two CCP from human C1r, both alone (CCP1 and CCP2) and in pair (CCP1-CCP2). My further goal was to characterize their internal dynamics and to figure out their flexible regions. I also aimed to determine the interaction surface of between the neighboring modules in the pair (CCP1-CCP2). Finally, I wished to outline the issue of complex formation between the two single modules.

3. Methods

I used primarily heteronuclear NMR-spectroscopic investigations and subsequent modeling. To reach the goals, single and pair of CCP modules were necessary to produce by biotechnological methods. The assignment of backbone atoms of the modules was achieved by proton-nitrogen correlated HSQC and 3D TOCSY-HSQC and NOESY-HSQC spectral analysis. Secondary $^{\alpha}H$ chemical shifts were mainly used for the analysis of secondary and tertiary structural analysis. The cross-peaks of HSQC spectra were compared to follow shift changes. The characterization of backbone model-free dynamics is based on the relaxation rates of HSQC-like measurements (T_1 and T_2) and also on determination of NOE enhancements by using TENSOR2 program. I used an in-house program for reduced spectral density analysis and the HYDRPRO for determination of the hydrodynamics behavior of the molecules based on publicly available crystal structures. Titration of the free modules is achieved in 10 steps. The residual atomic solvent accessible surface area was calculated by the crystal structures using the program NACCESS. The calculated data along with the titration results are applied to protein-protein docking by the HADDOCK program.

4. Results

1. I have carried out the assignment of ^{1}H and ^{15}N chemical shifts of the heteronuclear 2D and 3D spectra of the ^{15}N-labeled free CCP1, CCP2 and their covalently linked pair (CCP1-CCP2) at various temperatures, in acidic and neutral solutions. I pointed out that the solution structure is similar to the crystal structures.

2. Applying relaxation measurements (T_1, T_2 and heteronuclear NOE), I have determined backbone dynamics of CCP1, CCP2 and CCP1-CCP2. I appointed that the modular structures are considerably rigid. Significant mobility is defined at certain surface loops, namely the GH loop in CCP1 at the slow (μs-ms) and the EF in CCP2 at the rapid (ps-ns) time scale.

3. Rapid flexibility of EF loop in CCP2 is in conjunction with its low electron density in the crystal structures, thus with the poorly defined local structure, which shows therefore true flexibility.

4. Using chemical shift analyses, I have characterized the alterations between the free modules and the covalently linked pairs. The interaction between the modules was identified at Y325 and Y381. I raised that even if the two modules are distinct structural units, CCP1 requires the presence of one of its neighboring modules for its proper folding (CUB2), and for keeping its stability (CCP2).

5. Cross-titration experiments of CCP1 and CCP2 proved that the free modules are in weak interaction. The interaction was identified by alterations both in HN chemical shifts and in R_2 relaxation rates. The contact surface is different from that observed in the covalently linked pair. The interaction was modeled by protein-protein docking and based on this novel interaction a structural dimer was suggested for the catalytic fragment, which can plausibly be formed by small structural rearrangement from the modeled crystal structure, but its functional state is likely different.

5. Discussion

The crystal structure of C1r CCP molecules were already known at the beginning of the study. Nevertheless, these structures do not provide information on the internal dynamics of CCP modules, except the B-factor indirect values. The applied relaxation-dynamics studies of CCP1, CCP2 and CCP1-CCP2 introduced previously unknown behaviors of the modules.

In a structural point of view, the free modules are well comparable to the available structures of other CCP modules and also highly similar to the modeled CCP modules from crystal structure of the γ_B fragment. The suggested novel dimer may also contribute to the deeper insight of C1r function.

6. Publications

LÁNG A, GYÖRGY K, CSIZMADIA IG, PERCZEL A (2003): A conformational comparison of N- and C-protected methionine and N- and C-protected homocysteine. Journal of Molecular Structure-Theochem 666-667:219-241.

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LÁNG A, SZILÁGYI K, MAJOR B, GÁL P, ZÁVODSZKY P, PERCZEL A (2010): Interaction between separated consecutive complement control modules of human C1r: implications for dimerization of the full-length protease. FEBS Lett